Prejunctional auto- and heteroregulation of autonomic neurotransmission in the airways

de Haas, Jan Roelof Adrianus

Summary

The autonomic nervous system controls many aspects of airway function, including airway smooth muscle tone, mucus secretion, and intrapulmonary vascular permeability and blood flow. The predominant control is provided by parasympathetic nerves, using acetylcholine (ACh) as the neurotransmitter, to give bronchoconstriction, enhanced mucus secretion, and bronchial vasodilatation. Sympathetic innervation, using noradrenaline (NA) as the neurotransmitter, is generally much sparser. Sympathetic neurotransmission causes inhibition of mucus secretion, bronchial vasoconstriction, and relaxation of airway smooth muscle. In addition to these classic components of the autonomic nervous system, airways receive non-adrenergic non-cholinergic (NANC) input. This thesis focuses on the prejunctional auto- and heteroregulation of neurotransmitter release from cholinergic and adrenergic nerves innervating the airways. Electrical field stimulation (EFS)-induced simultaneous release of endogenous ACh and NA release from airway autonomic nerves were measured using two sensitive HPLC systems equipped with electrochemical detection. This method enabled us to study directly the control of neurotransmitter release via prejunctional muscarinic and adrenergic receptors in guinea pig as well as in human airway preparations.

In epithelium-denuded guinea pig tracheal section, EFS (biphasic square wave pulses, 16 Hz, 150 mA, 0.8 ms, for 5 min) evoked the release of ACh [29 pmol × (g tissue)$^{-1}$ × (5 min)$^{-1}$] and NA [70 pmol × (g tissue)$^{-1}$ × (5 min)$^{-1}$]. This release appeared under the *auto*inhibitory control of muscarinic and α_2-adrenergic receptors, respectively, as indicated by an increased ACh and NA release after incubation with the muscarinic and α_2-adrenoceptor antagonists atropine and yohimbine (**chapter 2**). In addition to this autoregulation, we established that endogenously released ACh and NA also controlled each other's release *via* prejunctional muscarinic and α_2-adrenergic *hetero*receptors. This was shown in experiments in which the blockade of prejunctional muscarinic receptors resulted in a clearly enhanced release of both ACh and NA. An adrenergic control of ACh release through α_2-adrenergic heteroreceptors was demonstrated by a yohimbine-induced augmentation of ACh release, but, only under conditions of elevated synaptic NA levels, *i.e.* after blocking NA reuptake with desipramine. Under our standard stimulation conditions prejunctional α_2-

adrenergic autoreceptors were maximally activated by endogenously released NA; only at lower stimulation frequencies (2-4 Hz) the α_2-adrenoceptor agonist clonidine was able to induce an inhibition of evoked ACh and NA release. The existence of prejunctional heteroregulation of neurotransmitter release might have clinical importance with respect to asthma, because the presumed dysfunction of muscarinic autoreceptors on cholinergic nerves, resulting in elevated ACh levels in asthmatic airways, could also lead to a reduction of NA release, shifting the smooth muscle contraction/relaxation balance even further to the contractile side.

In a subsequent study we investigated the functional implications of the prejunctional auto- and heteroregulation of neurotransmitter release as described above (**chapter 3**). EFS (biphasic square wave pulses, 16 Hz, 150 mA, 0.8 ms, for 4 sec with intervals of 80 sec) was used to elicit twitch contractions and relaxations of single epithelium-denuded open-ring preparations of guinea pig tracheae. The EFS-induced twitch contractions consisted solely of muscarinic M_3 receptor-mediated cholinergic responses, while relaxant responses mainly involved timolol-sensitive adrenergic neurotransmission (~70%) and inhibitory non-adrenergic non-cholinergic neurotransmission (~30%), sensitive to the nitric oxide synthase inhibitor L-NAME. In agreement with our release experiments, both the cholinergic twitch response and the adrenergic component of the relaxant response were under clear inhibitory control of prejunctional autoreceptors of the muscarinic M_2 and the α_2-adrenergic subtype, respectively. The α_2-adrenergic heteroreceptors played a minor role in the control of the cholinergic contraction. In contrast to release data, muscarinic M_2 heteroreceptors were not of any significance in the control of the NA- (and NO-) mediated component of the relaxation. This discrepancy may lead to the assumption that the muscarinic heteroreceptor in the guinea pig trachea is of pharmacologically distinct subtype (*i.e.*, more 'M_2-like' than classic M_2) with a lower affinity towards muscarinic M_2 receptor antagonists. There may also be threshold-effects in the activation of the heteroreceptor. The concentration of released ACh may exceed the threshold-activation-level of the muscarinic heteroreceptors in release but not functional experiments. In the presence of L-NAME, but not of timolol, the twitch contraction was significantly enhanced, indicating functional antagonism by NO, but not NA, of the cholinergic response. Another interesting finding was that blockade of muscarinic M_2 heteroreceptors

resulted in a strongly enhanced inhibitory function of α_2-adrenergic autoreceptors, as revealed by the exaggerated 2.5-fold potentiation of the relaxant response by yohimbine. This might indicate that these receptors share common G_i-proteins; blockade of one inhibitory receptor enhances the availability of G_i to the other inhibitory receptor.

The presence and function of prejunctional β_2-adrenoceptors in guinea pig airways were studied in **chapter 4**. Preincubation with the β_2-adrenoceptor agonist fenoterol resulted in a clear facilitation of EFS-evoked ACh and NA release, an effect that could be abolished completely by the β_2-adrenoceptor antagonist ICI 118,551. Prejunctional β_2-adrenoceptors on adrenergic nerve endings showed a strong facilitation of NA release (up to ~500% of control) that was concentration-dependent (0.1-100 μM fenoterol). The observation that the facilitatory effect diminished with longer agonist contact-times (1-15 min) indicated that β_2-adrenoceptors on adrenergic nerve endings are readily susceptible to desensitization. By contrast, sensitivity to fenoterol on cholinergic nerve terminals increased when the incubation period was prolonged; in addition, a bell-shaped concentration-response relationship was found at 15 min incubation. The results showed that β_2-adrenoceptors on cholinergic and adrenergic nerve endings possess different characteristics both with respect to their capacity to facilitate neurotransmitter release and their susceptibility to desensitization.

The differential facilitatory responses of prejunctional β_2-adrenoceptors on the two types of autonomic nerves as described above, indicated us to take a closer look at the signal transduction mechanisms of these receptors (**chapter 5**). To investigate the involvement of cyclic AMP (cAMP) in the β_2-adrenoceptor-mediated facilitation of neurotransmitter release, the direct activator of adenylyl cyclase, forskolin, was used. Forskolin (0.1-100 μM) induced a concentration-dependent augmentation of ACh release (to 195% of control), but not of NA release, to our surprise. This would indicate that, in contrast to cholinergic nerves, β_2-adrenoceptors on adrenergic nerve endings do not mediate their facilitatory effects *via* activation of the adenylyl cyclase pathway. As an alternative mode we considered a cAMP-independent activation of Ca^{2+} channels, since influx of Ca^{2+} ions is the actual trigger for exocytotic neurotransmitter release from nerve endings. In the presence of nifedipine, neither control release nor the fenoterol-induced facilitation of NA (and of ACh) release were changed,

indicating that L-type Ca^{2+} channels are not involved at all. When control release was diminished by preincubation with ω-conotoxin GVIA, a selective N-type Ca^{2+} channel antagonist, the capacity of fenoterol to facilitate NA release was markedly enhanced, surprisingly. At first sight, this would suggest the presence of an additional transduction mode of prejunctional β_2-adrenoceptors to facilitate neuronal Ca^{2+} influx (or mobilization) in adrenergic nerve terminals. However, when control NA release was dimished to a similar extent by lowering the stimulation frequency from 16 Hz to 4 Hz, the facilitation by fenoterol was also enhanced, by approximately the same factor. Collectively, the results suggest different signal transduction modes of prejunctional β_2-adrenoceptors to be operative in adrenergic and cholinergic nerve terminals.

Using tube preparations of human intrapulmonary bronchi, we were able to show for the first time that in addition to ACh [18 pmol × (g tissue)$^{-1}$ × (5 min)$^{-1}$], also endogenous NA [55 pmol × (g tissue)$^{-1}$ × (5 min)$^{-1}$] was released when the preparations were subjected to electrical field stimulation (**chapter 6**). Release of both neurotransmitters was inhibited after administration of 1 μM TTX, indicating the neuronal origin of both ACh and NA. Since β_2-adrenoceptor mediated facilitation has not been reported previously in human bronchi, we studied the putative prejunctional effects of fenoterol on the release of the two transmitters. Analogous to the results obtained with guinea pig tracheae, EFS-evoked release of both ACh *and* NA was facilitated after incubation with fenoterol (1-100 μM, 15 min preincubation). The highest concentration of fenoterol augmented ACh release to 148% of control, while the simultaneous release of NA was augmented to 185%. Preincubation with the β_2-adrenoceptor antagonist ICI 118,551 abolished the fenoterol-induced facilitation of both ACh and NA release. Although sympathetic nerves sparsely innervate human airway musculature directly, the released NA may originate from sympathetic nerves projecting to subepithelial blood vessels, bronchial glands and the blood vessels of these glands. A clinical implication of these findings might be that in addition to postjunctional airway smooth muscle relaxation, β_2-adrenoceptor agonists may reduce mucosal congestion by enhancing NA release from sympathetic nerve endings. These beneficial effects may overrule the adverse effect of enhanced ACh release from parasympathetic nerves.

The endogenous agonists for prejunctional β_2-adrenoceptors may be circulating adrenaline derived from the adrenal medulla and to some extent NA

released from local sympathetic nerves. In **chapter 7** we have investigated whether sympathetic nerves innervating the guinea pig trachea can take up, store, and release adrenaline as a co-transmitter of NA, and to which extent co-released adrenaline is able to facilitate the release of endogenous NA and ACh. Preincubation of the tissue with adrenaline (0.1-1.0 μM) followed by washing resulted in a desipramine-sensitive uptake and storage into, and in subsequent EFS-induced co-release of adrenaline from the sympathetic nerves. Initial release of adrenaline (up to 117% compared to control NA release) was always accompanied by a reduction in NA release, while total catecholamine release was enhanced. This increase could not be attributed to activation of prejunctional α_2- or β_2-adrenoceptors, respectively, since the patterns of adrenaline and NA release were not altered after yohimbine or ICI 118,551. Protection of the prejunctional β_2-adrenoceptors from putative desensitization during adrenaline-loading, using the hydrophilic β-adrenoceptor antagonist sotalol, did not further enhance catecholamine release. Combined with the data in Chapter 4 showing that prejunctional β_2-adrenoceptors with marked facilitatory capacity *are* present on sympathetic nerves of the guinea pig trachea, the results indicate that these receptors are not localized in close proximity to the exocytotic release sites of the varicosities. Evoked release of ACh was not affected by adrenaline preloading and subsequent EFS-induced co-release, indicating that adrenaline co-released from sympathetic nerves may not reach the prejunctional β_2-heteroreceptors on cholinergic nerves either. Nevertheless, the results suggest that due to enhanced half-life as a result of intraneuronal storage, adrenaline released as a co-transmitter of NA might increase the sympathetic control over those structures in the airways that receive adrenergic innervation.

MAIN CONCLUSIONS

The evoked release of ACh and NA from autonomic nerves in the guinea pig trachea is not only subject to prejunctional inhibitory *auto*regulation but also to inhibitory *hetero*regulation *via* α_2-adrenergic and muscarinic acetylcholine receptors. However, only the prejunctional muscarinic M_2 and α_2-adrenergic *auto*receptors control postjunctional cholinergic contractions and adrenergic relaxations to a major extent. Interestingly, autoregulation of

adrenergic relaxations by prejunctional α_2-receptors is under marked additional inhibitory control of M_2-heteroreceptors; in contrast, there is only a minor postjunctional role for prejunctional α_2-heteroreceptors.

In guinea pig trachea and human bronchus, evoked release of both ACh and NA is under facilitatory control of prejunctional β_2-adrenoceptors. Especially the receptors on adrenergic nerve endings have a strong capacity to facilitate NA release, which -in the guinea pig trachea- are subject to desensitization, however. The facilitatory effect of prejunctional β_2-adrenoceptors on cholinergic nerve endings is mediated through activation of the cAMP-cascade pathway, whereas the signaling in adrenergic nerve endings is cAMP-independent. In addition to postjunctionally mediated relaxation of airway smooth muscle, inhaled β_2-adrenoceptor agonists as well as adrenaline taken up into and released as a cotransmitter from sympathetic varicosities, may have bronchodilatory effects by enhancing sympathetic nerve output, consequently reducing mucosal congestion and edema.